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Nonmyeloablative Allogeneic Hematopoietic Stem Cell Transplantation for GATA2 Deficiency

Jennifer Grossman [1], Jennifer Cuellar-Rodriguez [2], Juan Gea-Banacloche [3], Christa Zerbe [2], Katherine Calvo [4], Thomas Hughes [5], Fran Hakim [3], Kristen Cole [3], Mark Parta [6,7], Alexandra Freeman [2], Steven M. Holland [2], Dennis D. Hickstein [3,*]

[1] *Division of Hematology and Hematologic Malignancies, Alberta Health Services, Calgary, Alberta, Canada*
[2] *Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland*
[3] *Experimental Transplantation and Immunology Branch, National Cancer Institute, Bethesda, Maryland*
[4] *Department of Laboratory Medicine, National Institutes of Health Clinical Center, Bethesda, Maryland*
[5] *Department of Pharmacy, National Institutes of Health Clinical Center, Bethesda, Maryland*
[6] *Leidos Biomedical Research, Inc, Frederick, Maryland*
[7] *Intramural Clinical Management & Operations Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland*

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A B S T R A C T

We treated 14 patients with GATA2 deficiency using a nonmyeloablative allogeneic hematopoietic stem cell transplantation regimen. Four patients received peripheral blood stem cells from matched related donors (MRD), 4 patients received peripheral blood stem cells from matched unrelated donors (URD), 4 patients received hematopoietic stem cells from umbilical cord blood donors (UCB), and 2 patients received bone marrow cells from haploidentical related donors. MRD and URD recipients received conditioning with 3 days of fludarabine and 200 cGy total body irradiation (TBI). Haploidentical related donor recipients and UCB recipients received cyclophosphamide and 2 additional days of fludarabine along with 200 cGY TBI. MRD, URD, and UCB recipients received tacrolimus and sirolimus for post-transplantation immunosuppression, whereas haploidentical recipients received high-dose cyclophosphamide followed by tacrolimus and mycophenolate mofetil. Eight patients are alive with reconstitution of the severely deficient monocyte, B cell, and natural killer cell populations and reversal of the clinical phenotype at a median follow-up of 3.5 years. Two patients (1 URD recipient and 1 UCB recipient) rejected the donor graft and 1 MRD recipient relapsed with myelodysplastic syndrome after transplantation. We are currently using a high-dose conditioning regimen with busulfan and fludarabine in patients with GATA2 deficiency to achieve more consistent engraftment and eradication of the malignant myeloid clones.

INTRODUCTION

Expression of the *GATA2* transcription factor is tightly regulated during hematopoiesis, and both over- and under-expression of GATA2 have been associated with myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) [1,2]. Recently, heterozygous sporadic or inherited mutations on 1 allele of the *GATA2* gene have been shown to be responsible for a syndrome known variably as MonoMAC (monocytopenia with nontuberculous mycobacterial infections (NTM)) [3]; dendritic, monocytes, and lymphoid cell deficiency [4]; Emberger's syndrome (congenital lymphedema with MDS and monosomy 7) [5]; and familial MDS/AML [2].

Allogeneic hematopoietic stem cell transplantation (HSCT) represents a potentially curative therapy for patients with GATA2 deficiency; however, these patients pose a particular therapeutic challenge because of the frequent comorbidities associated with this disease. These comorbidities include life-threatening infections secondary to deficiency of monocytes, natural killer (NK) cells, and B cells, as well as pulmonary alveolar proteinosis (PAP) resulting from defective alveolar macrophages [6,7]. In addition, the MDS in patients with GATA2 deficiency has the propensity to transform into AML or proliferative chronic myelomonocytic leukemia (CMML) [8].

We used a nonmyeloablative transplantation regimen to treat patients with GATA2 deficiency because the majority of

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* Correspondence and reprint requests: Dr. Dennis D. Hickstein, 9000 Rockville Pike, MSC1203, Building10/CRC, Rm 3-3142, Bethesda, MD 20892.
E-mail address: hicksted@mail.nih.gov (D.D. Hickstein).

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patients in our initial cohort had severe underlying organ dysfunction and active infections at the time of transplantation. In 2011, we published a pilot study describing encouraging results using a nonmyeloablative regimen in 6 patients with GATA2 deficiency [9]. Here, we describe the outcomes of the first 14 patients who received nonmyeloablative allogeneic HSCT for this newly described genetic syndrome, including the first 2 patients to receive a haploidentical transplant.

PATIENTS AND METHODS

Patients

This study was designed to determine the efficacy and safety of nonmyeloablative allogeneic HSCT for patients with GATA2 deficiency, or MonoMAC syndrome, and was approved by the institutional review board of the National Cancer Institute. This study was independently monitored for safety and data accuracy (ClinicalTrials.gov number, NCT00923364). Patients between 12 to 60 years of age were eligible if they met the following criteria: (1) at least 2 episodes of life-threatening opportunistic infections; (2) mutation in the *GATA2* gene, or a flow cytometry profile on peripheral blood demonstrating severe monocytopenia and $CD19^+$ B cell and $CD3^-CD56^+$ NK cell lymphopenia, consistent with the MonoMAC phenotype [10]; and (3) a 10/10 or 9/10 matched related donor (MRD) or matched unrelated donor (URD), a 4/6 or greater matched umbilical cord blood donor (UCB), or a haploidentical related donor.

Patients were allowed to have MDS with 1 or more peripheral blood cytopenias and less than 5% blasts in the bone marrow in the absence of granulocyte colony–stimulating factor.

Characteristics of Allogeneic HSCT

MRD and URD were required to be 18 years of age or older and matched at 10/10 or 9/10 HLA-A, -B, -C, -DRB1, and -DQB1 loci by high-resolution typing [11]. In addition, MRD and haploidentical related donors were required to have either a normal *GATA2* gene on DNA sequencing or normal blood monocyte, NK cell, and B cell counts, and no history of mycobacterial or any other opportunistic infections.

Haploidentical related donors were required to share 1 haplotype in common with the recipient, such that HLA compatibility represented a minimum match of 5 out of 10 HLA loci. As with MRDs, exclusion criteria included mutation in *GATA2*, history of mycobacterial or other opportunistic infections, or abnormal monocyte, NK, or B cell counts. If more than 1 haploidentical donor were available, each donor was evaluated individually in terms of overall health, ABO matching, and cytomegalovirus (CMV) status, etc. to select the best donor.

The conditioning regimen depended upon the type of donor graft. MRD or URD recipients received fludarabine 30 mg/m^2/day on days −4, −3, and −2, and 200 cGy total body irradiation (TBI) on day −1. UCB recipients received cyclophosphamide 50 mg/kg i.v. on day −6, fludarabine 30 mg/m^2/day i.v. for 5 days (days −6 to −2), and 200 cGy TBI on day -1. Haploidentical related donor recipients received fludarabine 30 mg/m^2/day i.v. for 5 days (days −6 to −2), cyclophosphamide 14.5 mg/kg i.v. for 2 days (days −6 and −5), and 200 cGy TBI on day −1 [12].

The cell product also depended upon the type of transplant. MRD and URD received peripheral blood stem cells from their donors obtained using 5 days of granulocyte colony–stimulating factor (filgrastim) (10 μg/kg/day) followed by apheresis with the goal of collecting at least 5×10^6 $CD34^+$ cells/kg of the recipient's body weight. MRD and URD peripheral blood stem cells were infused fresh on day 0. Umbilical cord blood units were thawed and infused on day 0. Haploidentical related donor recipients received fresh or cryopreserved bone marrow cells on day 0, with a target dose of 2×10^8 total nucleated cells per body weight of the recipient. Graft-versus-host-disease (GVHD) prophylaxis was dependent upon the type of transplant. Tacrolimus and sirolimus were initiated on days −3 and −2, respectively, in MRD, URD, and UCB recipients. The doses of both agents were titrated to achieve serum levels between 5 and 10 ng/mL. Immunosuppression was tapered at 6 months after transplantation if there were no evidence of GVHD. Post-transplantation immunosuppression for haploidentical recipients consisted of cyclophosphamide 50 mg/kg/day i.v. for 2 days on days +3 and +4 followed by tacrolimus and mycophenolate mofetil starting on day +5 [12]. Immunosuppression with mycophenolate was stopped at day +30, and immunosuppression with tacrolimus was tapered at 6 months after transplantation if there were no evidence of GVHD.

Supportive Care

Standard guidelines for supportive care established at the National Institutes of Health Clinical Center for patients undergoing allogeneic HSCT were used. These guidelines are in agreement with the international guidelines for preventing infectious complications among hematopoietic cell transplantation recipients [13].

For NTM infections, infectious disease physicians with expertise in therapy for NTM infection were involved in the care of these patients. Treatment selection was based on the specific NTM species isolated [14]. When patients had their NTM infection fully treated before transplantation, they were kept on prophylactic azithromycin until 1 year after transplantation. In cases in which the NTM infection was recent, all efforts were made to delay transplantation until the NTM infection was under control, cultures became negative, and lesions were stable or regressing on imaging studies. For patients whose infection episodes were recent or had bone involvement and were still on a rifamycin as part of their treatment, this was changed to moxifloxacin before the start of the conditioning regimen, to avoid possible drug interactions. All patients had a macrolide as part of their treatment regimen or secondary prophylaxis; in all cases, azithromycin was selected over clarithromycin to avoid possible drug interactions. Most patients who were still being treated for active infection at the time of transplantation (double or triple therapy) were kept on all the antimycobacterial drugs at least 6 to 12 months after the transplantation. Subsequently, these were discontinued, and the azithromycin was kept for at least 6 more months.

Immune Reconstitution of T, B, and NK Cells and Monocytes

$CD14^+$ monocytes, $CD3^-/CD56^+$ NK cells, $CD19^+$ B lymphocytes, and $CD3^+$ T lymphocytes were quantified by flow cytometry using subset specific monoclonal antibodies before transplantation and at designated intervals after transplantation.

Cytogenetics

Cytogenetic analysis was performed before transplantation and at 28 days and 12 months after transplantation. When cytogenetic abnormalities were present, fluorescence in situ hybridization was used to identify the specific chromosomal abnormalities.

Analysis of Chimerism

Engraftment of donor cells was assessed using polymorphisms in regions known to contain short tandem repeats. Peripheral blood $CD14^+$, $CD3^-/CD56^+$, $CD19^+$, and $CD3^+$ subsets were isolated by flow cytometry at designated time points, and chimerism was assessed. In addition, $CD14^+/CD15^+$ myeloid cells and $CD3^+$ T lymphocytes were selected using immunobeads and chimerism was assessed on the selected cells.

Statistical Analysis

Descriptive statistics were used for chimerism, monocyte, NK, and lymphocyte counts.

RESULTS

Study Population

Baseline characteristics of the 14 patients with mutations in *GATA2,* or MonoMAC syndrome, who received allogeneic stem cell transplantation are shown in Table 1. The median age at the time of transplantation was 33 years (range, 15 to 46 years). The majority of patients had suffered infections since childhood. The median duration of illness before transplantation was 7.5 years (range, 1 to 28 years). All patients had characteristic flow cytometry findings in their peripheral blood with severely reduced $CD14^+$ monocytes, $CD19^+$ B lymphocytes, and $CD3^-/CD56^+$ NK cells, along with normal to slightly reduced $CD3^+$ T lymphocytes.

Susceptibility to opportunistic infections, especially with NTM mycobacteria species, is a hallmark of MonoMAC syndrome, and all but 2 patients had a history of an NTM infection (Table 1). Severe DNA viral infections, most frequently human papillomavirus, were common, presumably due to the lack of NK cells. One patient had a severe refractory human herpes simplex virus infection. Over one half of the patients in our cohort had serious fungal and/or bacterial infections before transplantation.

Pulmonary disease was also common in this cohort with 5 patients having PAP (Table 1). Two patients were on supplemental oxygen before transplantation because of this

Table 1
Characteristics of Patients with GATA2 Deficiency Receiving HSCT

Donor	Patient	Age at HSCT, yr/sex	Duration of Illness, yr	Type of Infection			Pulmonary Manifestations	Mutation	Family History*
				NTM	Viral	Other			
MRD	1	33/M	24	Disseminated MAI	Skin/genital HPV	Invasive aspergillosis	PAP	delAinsGC G81fs	-
	2	46/F	28	Disseminated MAI, *Mycobacterium scrofulaceum, M. fortuitum*	Skin HPV, VZV	Disseminated Aspergillosis	PAP	R398W	+*
	3	45/F	2	Disseminated MAI	VZV	-	Ground glass and reticular infiltrates	Exon 6 skip	-
	4	33/M	5	Disseminated MAI	Anal and genital HPV	-	PAP	T354M	+*
URD	5	33/M	1	Disseminated MAI	Skin HPV; molluscum contagiosum	-	-	R398W	+*
	6	23/F	8	Disseminated *M. abscessus*		Disseminated Nocardiosis	Reticular infiltrates/bullae	R398W	-
	7	38/F	26	MAI	HPV genital, disseminated VZV	Culture (-) endocarditis, *C. difficile* toxic megacolon	Ground glass and reticular infiltrates	N371K	Adopted
	8	33/M	13	Granulomatous adenitis	-	Necrotizing fasciitis	Ground glass and reticular infiltrates/bullae	T354M	+*
UCB	9	41/F	22	Disseminated MAI and *M. abscessus*	Skin and genital HPV	-	PAP	T354M	+*
	10	15/M	2	Disseminated MAI	Skin HPV	*S. sanguinis* osteo, *C. difficile* toxic megacolon	Reticular and nodular infiltrates/bullae	Ins 10bp D259fs	-
	11	29/M	16	*M. kansasii*	Skin and genital HPV	VRE empyema, Candida sinovitis, *C. difficile*	Ground glass and reticular infiltrates	R361del14	-
	12	27/F	2	Disseminated *M. kansasii*	None	-	PAP	A318fs	-
Haplo	13	22/F	7	None	Genital HPV, refractory genital HSV, disseminated CMV	-	Ground glass infiltrates/bullae	G101fs	-
	14	20/F	1	Disseminated MAI	Hydroa vaccineforme-like EBV+ T cell lymphoma	Histoplasmosis	Reticular infiltrates	MonoMAC syndrome†	-

MAI indicates *mycobacterium avium intracellulare*; M, male; HPV, human papillomavirus; F, female; VZV, varicella zoster virus; VRE, vancomycin-resistant enterococcus; Haplo, haploidentical related donor; HSV, human herpes simplex virus; EBV, Epstein-Barr virus.

* Specifics of family history: patient 2, sister died at age 31 after HSCT and mother died at age 54 of CMML; patient 4, cousin had leukemia and 2 sons with the same mutation but asymptomatic thus far age 3 and 10; patient 5, mother died at age 32 of AML; patient 8, father, aunt, and uncle had AML; patient 9, son died age at 19 of AML.

† No identifiable mutation in *GATA2*, but expressed only the paternal allele of *GATA2* at the mRNA level.

condition. The severity of the disease is evidenced by the fact that 1 patient (patient 1) was considered to be a lung transplantation candidate before HSCT and ultimately received a double lung transplant 4 years after HSCT. In addition, patient 2 underwent transplantation while intubated after pretransplantation whole lung lavage for severe PAP.

The patients in this study had a variety of mutations in *GATA2*, the 2 most common being T354M and R398W (Table 1). Patient 14 did not have an identifiable mutation in an exon of *GATA2* but expressed only the paternal allele of *GATA2* at the mRNA level. Five patients had a parent or sibling with a *GATA2* mutation, and the remaining 9 patients did not have a family history of the syndrome in a first-degree relative.

All patients had bone marrow involvement, with 12 patients (86%) meeting World Health Organization criteria for MDS. Most patients had refractory cytopenia with multilineage dysplasia at the time of diagnosis. Two patients had progressed to refractory anemia with excess blasts before transplantation (Table 2). In contrast to patients with typical MDS, the majority of patients had bone marrow biopsies that were hypocellular with reticulin fibrosis. On cytogenetic analysis, 4 patients had monosomy 7, an abnormality known to be associated with a poor prognosis in MDS and AML (Table 2).

The typical progression of the bone marrow histology in GATA2 deficiency is shown (patient 7) (Figure 1). Approximately 2 years before transplantation, her marrow was hypocellular with reduced granulocyte precursors and monocytes and normal cytogenetics (Figure 1A,B). Over a 2-year period, her marrow became hypercellular with 5% to 7% blasts, consistent with a diagnosis of refractory anemia with excess blasts−1, and cytogenetic analysis revealed a new monosomy 7 (Figure 1C,D). Six months after induction chemotherapy and an unrelated donor transplantation, the bone marrow histology and cytogenetic analysis were normal (Figure 1E,F).

Pretransplantation chemotherapy was required in 3 patients. Two patients (patients 7 and 9) required 1 cycle of chemotherapy with idarubicin and cytosine arabinoside (7 + 3) before transplantation because the bone marrow blast count was greater than 5% (Table 2). One patient (patient 13) presented with an MDS/myeloproliferative syndrome (CMML) and received 3 cycles of chemotherapy (7 + 3, then high-dose cytosine arabinoside and melphalan) before transplantation. This patient had received chemotherapy for AML 7 years earlier and had achieved a complete remission.

Transplantation Characteristics

This cohort of patients received a cross section of graft sources with 4 MRD, 4 URD, 4 UCB, and 2 haploidentical related donors. Peripheral blood stem cells were used in all MRD and URD donors, and bone marrow was used for haploidentical related donor recipients. Cell doses were comparable in the MRD, URD, and haploidentical related donor groups (Table 3).

Reconstitution of Hematopoietic Compartments after Transplantation

Reconstitution of the cellular compartments that were severely deficient before transplantation represented a primary objective of this study. The median time to neutrophil engraftment (defined as a neutrophil count of $>.5 \times 10^9$ cells/L for 3 consecutive days) for recipients of MRD and URD was 12 days (range, 0 to 13 days). The 3 evaluable patients in the UCB group engrafted neutrophils within a range from 16 to 80 days. Of the 2 haploidentical transplant recipients, the first patient died early after transplantation and the second patient had neutrophil engraftment by day 19 after

Table 2
Reversal of MDS and Cytogenetic Abnormalities after HSCT

Donor	Patient	Pretransplantation Bone Marrow			Pretransplantation Treatment	Two-year Post-transplantation Bone Marrow		
		Diagnosis	Cytogenetics	Cellularity*		Diagnosis	Cytogenetics	Cellularity*
MRD	1	RCMD	Normal	Hypocellular	None	Trilineage hematopoiesis	46, XY	Normocellular
	2	RCMD	N/A	Hypocellular	None	ND	ND	ND
	3	RCMD	Trisomy 1q	Hypocellular Gr 1-2 Fibrosis	None	Trilineage hematopoiesis	46, XY	Hypocellular Gr 1 fibrosis
	4	RCMD	Trisomy 8	Hypocellular Gr 1-2 Fibrosis	None	Trilineage hematopoiesis	46, XX	Hypocellular Gr 1-2 fibrosis
URD	5	RCMD	-Y	Hypocellular Gr 2 Fibrosis	None	†Trilineage hematopoiesis	46, XX	Normocellular Gr 2 fibrosis
	6	RCMD	Trisomy 8	Hypocellular Gr 1-2 Fibrosis	None	Trilineage hematopoiesis	46, XX	Hypocellular Gr 1-2 fibrosis
	7	RAEB1	Monosomy 7	Hypercellular	3 + 7	Trilineage hematopoiesis	46, XX	Normocellular Gr 1 fibrosis
		RCMD	Trisomy 8	Hypocellular	None	ND	ND	ND
UCB	9	RAEB2	Monosomy 6, +r	Normocellular Gr 3 Fibrosis	3 + 7	Trilineage hematopoiesis	46, XY	Hypocellular Gr 3-4 fibrosis
	10	RCMD	Monosomy 7	Hypocellular	None	Trilineage hematopoiesis	46, XY	Hypocellular
	11	RCMD	Monosomy 7, Trisomy 21	Hypocellular Gr 2 Fibrosis	None	ND	ND	ND
	12	RCMD	Monosomy 6	Hypocellular Gr 2 Fibrosis	None	ND	ND	ND
Haplo	13	CMML	Monosomy -7q Trisomy 8	Hypercellular Gr 2 Fibrosis	HIDAC, Melphalan	ND	ND	ND
	14	HLH, EBV (+) T cell LPD	Normal	Hypocellular	Alemtuzumab, etoposide (HLH)	†Trilineage hematopoiesis, no EBV detected	N/A	Hypocellular

RCMD indicates refractory cytopenia with multilineage dysplasia; ND, not done as patient died before 2 years; Gr, grade; RAEB, refractory anemia with excess blasts; HIDAC, high-dose cytosine arabinoside; HLH, hemophagocytic lymphohistiocytosis; LPD, lymphoproliferative disorder; N/A, not available.

* Cellularity for age (100% - age ± 10%)

† Results of last available marrow. Patient 5 refused bone marrow biopsies as of 6 months after transplantation. Patient 9 is only 9 months after transplantation.

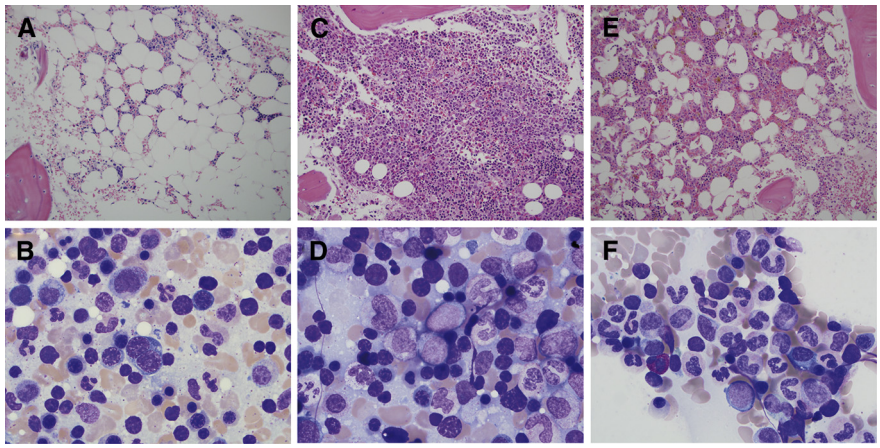

Figure 1. Typical evolution of pathologic changes in the bone marrow in GATA2 deficiency. Bone marrow findings for patient 7. (A), (C), and (E) show bone marrow biopsy results 2 years before transplantation, 2 months before transplantation, and 6 months after transplantation respectively. (B), (D), and (F) show bone marrow aspirates from the same time points. This demonstrates the progression of the bone marrow from hypocellular to hypercellular and finally to normal trilineage hematopoiesis.

transplantation. The pattern was similar for platelet engraftment (defined as a platelet count of $>20 \times 10^9$ cells/L for 7 consecutive days without requiring platelet transfusion). MRD recipients engrafted at a median of 16 days (range, 0 to 18 days), URD at 13 days (range, 0 to 18 days), the 2 evaluable UCB recipients engrafted at 32 and 302 days, and the single, evaluable haploidentical related donor recipient never had a platelet count below 20.

In the evaluable patients who initially engrafted, the percentage of donor chimerism at day 100 and at 1 year is shown (Figure 2). All evaluable patients achieved 100% donor myeloid cells by day +100 and 98% to 100% at 1 year. $CD14^+$ monocytes and $CD3^-/CD56^+$ NK cells were between 98% and 100% at both day +100 and 1 year. The number of $CD3^+$ cells at day +100 ranged from 29% to 100%, with a median of 91%. At 1 year, the range was 65% to 100%, with a median of 93%.

Table 3
Characteristics of Hematopoietic Stem Cell Grafts and Outcome of HSCT

Donor	Patient	Cell Source	HLA Match	Composition of Donor Graft		Infections after HSCT	GVHD		Outcome
				$CD34^+ \times 10^6$/kg	$CD3^+ \times 10^6$/kg		Acute	Chronic*	
MRD	1	PBSC	10/10	7.0	247	CRBSI	Gr II skin, liver	Severe	Alive 4.7 yr
	2	PBSC	10/10	4.1	380	IFI, CMV viremia, BSI	Gr IV skin, liver	N/A	Died d 90: sepsis, GVHD
	3	PBSC	10/10	6.0	174	-	Gr II skin, gut	-	Alive 3.2 yr, relapsed 1.4 yr, now 1.8 yr after second HSCT
	4	PBSC	10/10	6.4	330	-	-	Mild	Alive at 2.3 yr
URD	5	PBSC	10/10	6.87	354	-	-	-	Alive 4.5 yr
	6	PBSC	10/10	8.25	207	-	Gr III skin, gut, liver	Mild	Alive 3.5 yr
	7	PBSC	9/10	7.25	447	-	-	-	Alive 2.3 yr
	8	PBSC	10/10	6.3	188	CMV, IFI	Gr IV skin, gut, liver	-	Rejected 10 mo, underwent retransplantation, died at 1.5 yr
UCB	9	Single UC	4/6	.78	3.48	CRBSI, IFI, CMV-viremia	-	-	Developed donor cell AML 2.5 yr after HSCT and died at 3 yr
	10	Double				FN, endocarditis	Gr I-II skin, gut	-	Alive 3.4 yr
		UC1	4/6	.18	5.04				
		UC2	4/6	.65	10.5				
	11	Double				VRE sepsis, candida sepsis	N/A	N/A	Died d 7: sepsis
		UC1	4/6	.23	6.8				
		UC2	4/6						
	12	Double				VRE sepsis	Gr I-II gut	-	Rejected, underwent retransplantion, died d 295
		UC1	4/6	.16	ND				
		UC2	4/6	.57	ND				
Haplo	13	BM	5/10	4.2	43.2	ARDS	N/A	N/A	Died d 1: ARDS
	14	BM	7/10	6	55	*Aeromonas* sp. gastroenteritis	Gr I skin		Alive 8 mo

PBSC indicates peripheral blood stem cells; CRBI, catheter-related blood stream infection; IFI, invasive fungal infection; BSI, bloodstream infection; N/A, not applicable as patient died early after transplantation; UC, umbilical cord; FN, febrile neutropenia; BM, bone marrow; ND, not done; ARDS, acute respiratory distress syndrome.

* According to National Institutes of Health criteria for severity.

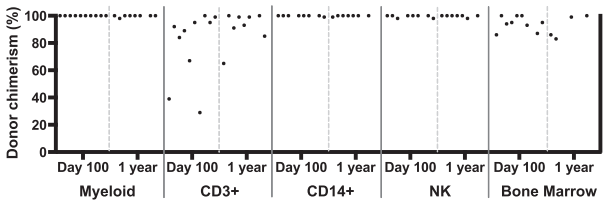

Figure 2. Chimerism after HSCT. Percentage of peripheral blood donor chimerism from myeloid, $CD3^+$, $CD14^+$, NK cell fractions, and bone marrow.

Of the 2 patients with low CD3 chimerism at day +100, the patient with the lowest chimerism (29%) (patient 8) subsequently rejected the unrelated donor graft and required retransplantation. The patient with 39% donor CD3 chimerism at day +100 (patient 1) maintained stable engraftment and ultimately progressed to 100% donor CD3 chimerism at 2 years after transplantation.

The reconstitution of cell subsets for a single GATA2 patient before transplantation and at 6 months after transplantation is shown (Figure 3).

Clinical Outcome after HSCT

Eight of the 14 (57%) patients in this high-risk cohort of patients are alive at a median follow-up of 3.5 years (range, 12 months to 5 years) (Figure 4). The overall survival based on donor type is shown (Figure 4). Mortality was not uniformly distributed in the subgroups: 3 of the 4 recipients in both the MRD and URD groups are alive, and 1 of the 2 haploidentical related donor recipients is alive. However, only 1 of the 4 UCB recipients survived (Figure 4). Of note, 5 of the 8 survivors (patients 1, 4, 5, 6, 7) had uneventful hospitalizations and were discharged within 1 month of transplantation.

In the MRD group, where all 3 survivors have greater than 2 years of follow-up, all patients engrafted with the only death occurring in the individual on a ventilator at the time of transplantation (patient 2). However, 1 patient (patient 3) relapsed 1 year after transplantation and required a second transplantation using the same sibling donor, preceded by a myeloablative regimen with busulfan and fludarabine. This patient remains in complete remission 2 years after retransplantation. A second patient (patient 1), who was a lung transplantation candidate before HSCT because of severe pulmonary hypertension and PAP, initially had marked improvement of his lung disease. However, he subsequently developed bronchiolitis obliterans and required lung transplantation 4.5 years after HSCT.

In the URD cohort, 3 of the 4 patients are alive with follow-up greater than 2 years (Figure 4). The only death occurred in a patient (patient 8) who rejected the URD graft 8 months after transplantation. He underwent retransplantation from a second URD, developed severe GVHD, and died.

The UCB group was the most problematic, with only 1 of 4 patients surviving long-term (Figure 4, Table 3). One patient (patient 11) died early from sepsis, another patient (patient 10) rejected the double cord graft and died after a salvage $CD34^+$-selected haploidentical related donor and single cord transplantation. The third patient in this group (patient 9) received a single UCB unit, had delayed engraftment, and did well for the subsequent 2 years, but ultimately died 2.5 years after transplantation from a donor cell–derived AML. The only long-term survivor in this cohort (patient 6) developed autoimmune nephrotic syndrome and pancytopenia on day 90, which ultimately responded to treatment with rituximab and tacrolimus.

In the haploidentical related donor cohort, 1 of the 2 patients (patient 14) is alive 9 months after transplantation. The first patient in this group had progressed to a proliferative CMML before transplantation, required multiple rounds of chemotherapy, and had multiple ongoing refractory infections. She died from respiratory failure on day +1. The second patient had recurrent gastrointestinal bleeding from an Epstein-Barr virus–positive T cell lymphoma that involved her gastrointestinal tract, skin, and lungs and associated hemophagocytic lymphohistiocytosis. She received a haploidentical transplantation from her sister and had marked resolution of the lymphoma, including extensive hydroa vacciniforme-like lesions.

Post-transplantation Complications

We anticipated major infectious complications in this group of immunocompromised patients. Nine patients (65%) had infections after transplantation (Table 3) and 3 deaths were attributable to infection. Overall, the most common pathogens were bacteria. Three patients had CMV reactivation (but no invasive disease) and 3 had invasive fungal infections. Surprisingly, none of the patients had reactivation of their NTM infections after transplantation; however, all patients with NTM were maintained on antimycobacterial therapy throughout the conditioning regimen and for at least 3 months after transplantation.

Eight (57%) of the 14 patients had acute GVHD, most commonly affecting the skin (Table 3). Three patients had grade III or IV GVHD affecting 2 or more organs. Acute GVHD was successfully treated with steroids (topical or systemic) in all but patient 2, whose death at 90 days was attributed in part to severe GVHD, and in patient 8 who developed severe GVHD after his second transplantation. Acute GVHD was most common in the MRD group. Chronic GVHD developed in 3 patients (1, 4, and 6), but was only severe enough to limit daily activities in 1 patient.

The baseline cytogenetic abnormalities resolved in all patients except the patient who relapsed and required a second transplantation (Table 2). This is particularly relevant in view of the adverse cytogenetics, with monosomy 7 present in 5 patients.

Although the groups are too small to derive statistically significant conclusions, there is a trend towards worse outcome with UCB grafts relative to the URD and MRD groups, where 75% of patients are still alive.

DISCUSSION

We report the outcome in a cohort of patients with GATA2 deficiency who received MRD, URD, UCB, or haploidentical related donor hematopoietic stem cells after a nonmyeloablative conditioning regimen. In patients with GATA2 deficiency, nonmyeloablative allogeneic HSCT results in reconstitution of the severely deficient monocyte, B, and NK cell populations, correction of the infection susceptibility phenotype, and reversal of the propensity for myeloid progression, including eradication of the abnormal cytogenetic clones. However, the incidence of graft rejection and relapse after nonmyeloablative allogeneic HSCT in GATA2 deficiency indicates that alternative transplantation approaches may be required.

Recently, deficits in the expression of *GATA2*, a "master regulator" of hematopoiesis, have been shown to lead to

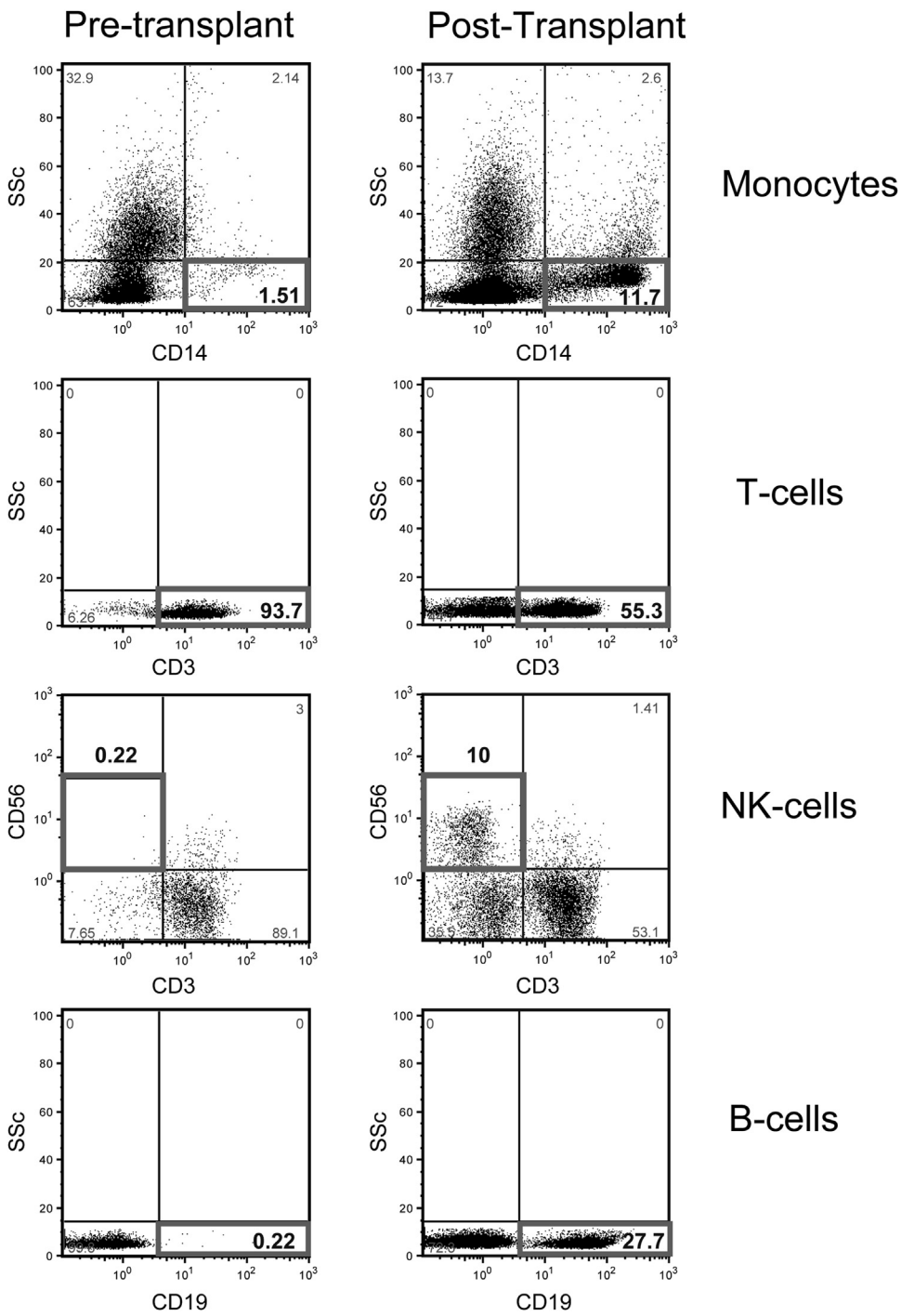

Figure 3. Reconstitution of CD19$^+$ B cells, NK cells, and monocytes 6 months after transplantation in a GATA2 deficient patient.

opportunistic infections, myeloid malignancies, and PAP [2–5]. Although HSCT represents the only definitive therapy for this disease, the indications for transplantation, optimal conditioning regimen, timing of transplantation, and donor source of stem cells remain unclear. In addition, the decision to undertake allogeneic transplantation has to be weighed against the complications inherent to HSCT, including regimen-related toxicity, GVHD, infection, and death.

The development of progressive bone marrow dysplasia and cytogenetic abnormalities represents 1 of the primary indications for allogeneic HSCT in GATA2 deficiency. *GATA2* is crucial for the maintenance of hematopoietic stem cells in that both mice and humans with GATA2 deficiency lack multilineage progenitors and have a severe depletion of granulocyte-macrophage progenitors in the bone marrow [15–17]. This defect in cell production typically evolves into a hypocellular MDS and ultimately progresses to AML or a proliferative CMML.

The largest series published on patients with mutations in *GATA2* found that 84% of patients with GATA2 deficiency met criteria for MDS on bone marrow biopsy [6]. Similarly, a high frequency of *GATA2* mutations was reported in 14

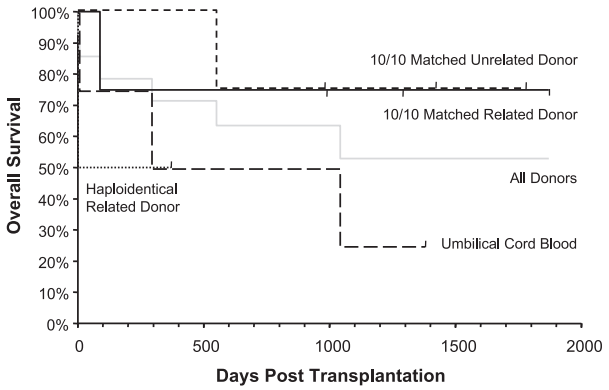

Figure 4. Kaplan-Meier curves showing overall survival according to type of donor.

patients in the French Severe Chronic Neutropenia Registry [15]. In this cohort, the risk of malignant transformation was high with 6 of the 14 patients developing refractory cytopenia with multilineage dysplasia and 4 developing AML. The median age at diagnosis of MDS or AML was 15 years (range, 7 to 35). Of note, none of the patients treated with chemotherapy alone survived (median survival of 1.5 years after diagnosis of AML).

The majority of patients with GATA2 deficiency have low or intermediate-1 International Prognostic Scoring System (IPSS) scores at the time of diagnosis [16,17]. All 14 of the patients in this study had low or intermediate-1 IPSS scores when diagnosed. According to the IPSS, the time for 25% of low-risk MDS patients to transform to AML is 9.4 years [16]. However, in our cohort, 2 of 4 patients with low-risk IPSS scores had a rapid transformation from a hypocellular MDS to a hypercellular MDS/refractory anemia with excess blasts. Thus, once the diagnosis of GATA2 deficiency is established, close follow-up of these patients is warranted, and patients should be considered for transplantation even before the appearance of blasts in their bone marrow.

The development of recurrent severe infections is a second indication to proceed to allogeneic HSCT. A subset of patients with mutations in *GATA2* present in their second or third decade of life with recurrent life-threatening opportunistic infections (frequently NTM), but they lack features of a malignant hematologic disease [6]. The mortality associated with these infections is enough to warrant replacement of the dysfunctional and deficient immune system. This is exemplified in 2 20-year-old patients in our study who died awaiting transplantation within the preceding year: 1 patient died from sepsis and 1 died from CMV pneumonia (D. Hickstein, unpublished data).

Progressive lung injury from infection and PAP represents a third reason for allogeneic HSCT in patients with GATA2 deficiency. Both frequent pneumonias as well as PAP result in gradual deterioration of lung function. The first 2 patients who underwent transplantation on this protocol had severe PAP requiring pulmonary lavage before transplantation, and both required supplemental oxygen at the time of transplantation. As mentioned previously, 1 patient has gone on to require bilateral lung transplantation and the other died early after transplantation. Therefore, initiating allogeneic HSCT before PAP develops would be anticipated to result in lower post-transplantation morbidity.

The ideal pretransplantation conditioning regimen for GATA2 deficiency is unclear. Moreover, it is likely that the stage of the disease at the time of transplantation may influence the type of conditioning. This study was initiated before the identification of the *GATA2* gene as the cause of MonoMAC syndrome, and all of the initial patients had considerable comorbidities necessitating a nonmyeloablative approach to allogeneic HSCT in this cohort. However, relapse in 1 of the 4 MRD recipients and graft rejection in 1 of the 4 recipients from each of the URD and UBC groups support the use of a more intensive conditioning regimen. Also, 3 patients required pretransplantation chemotherapy, which may have been unnecessary if a myeloablative regimen were used.

The timing of HSCT is well defined for many malignancies; however, the timing of allotransplantation is less clear for many immune deficiencies. GATA2 deficiency is particularly difficult, given its variable natural history with some patients developing symptoms only after many decades. However, after symptoms develop, survival declines [6]. The uninterrupted progression of GATA2 deficiency that we have seen suggests that the ideal time for transplantation would be after the onset of pathologic abnormalities, but before the development of organ damage or malignancy. Identifying more precise indicators of disease progression is urgently needed.

In this regard, we are currently investigating markers that portend myeloid progression. For example, it is well known that acquired mutations lead to poor-risk MDS/AML. To identify acquired somatic mutations associated with myeloid transformation in patients with GATA2 deficiency, we sequenced the region of the *ASXL1* gene previously shown to be associated with transformation from MDS to AML [8,17]. Heterogeneous somatic *ASXL1* mutations were identified in nearly 30% of patients, including 4 out of 5 patients who developed a proliferative CMML. Patients with *GATA2* and *ASXL1* mutations were considerably younger and almost exclusively female compared with patients with MDS and *ASXL1* alone [8,17].

Donor source remains a critical variable in the outcome of HSCT and this is evident in our study. MRD and URD transplantations were associated with the best outcomes, with 75% survival in both groups. The poor outcome with UCB supports the use of alternative donor sources, such as haploidentical related donors in cases where a 10/10-matched donor is not available. The rapid reconstitution of the deficient compartments is essential to clear the ongoing infections that affect many of the patients at the time of transplantation. As such, the prolonged time to engraftment with UCB transplantation is especially detrimental in this population.

Previously, patients with GATA2 deficiency underwent transplantation without prior knowledge of the genetic defect. Bigley et al. described 2 patients ages 12 and 21 who received URD transplants for GATA2 deficiency and both were alive 2 years after transplantation [17]. In a report of patients with Emberger's syndrome, 4 young patients (ages 9, 11, 12, and 16) received HSCT, primarily from URD, and only 1 survived [18]. In contrast, all 6 of the 14 patients in the French registry study who developed MDS or AML and underwent allotransplantation were alive at a median of 2 years after transplantation [15]. Our pilot study in 2011 described encouraging results using a nonmyeloablative regimen in 6 patients with GATA2 deficiency [9]. However, with further follow-up of the original cohort, and the inclusion of 8 additional patients, it now appears that more intensive conditioning is indicated in patients with GATA2 deficiency. Lastly, our overall mortality (42%) in this high-risk group of

patients, though heavily skewed by the poor outcome of UCB recipients, is comparable to recently reported transplantation outcomes [19].

In summary, although nonmyeloablative HSCT results in reversal of the hematologic, immunologic, and clinical manifestations of GATA2 deficiency, more uniform engraftment and a reduced risk of relapse would be anticipated with a more intensive conditioning regimen. To this end, we are now using a myeloablative regimen with busulfan and fludarabine for patients with GATA2 deficiency [20,21]. The optimal time to transplantation appears to be during the hypocellular MDS phase and before significant organ dysfunction develops. We anticipate that with the increasing frequency of genetic testing for *GATA2* mutations, patients will undergo transplantation earlier in the course of the disease, before significant organ damage or clonal evolution of MDS to AML or CMML occurs, and that the outcome of allogeneic HSCT in these patients will continue to improve.

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